together in TIF and further elucidates Caspase-1 activation is an upstream event of apoptotic Caspase-7 induction during TIF and TEC transdifferentition and apoptosis.

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Effect of Hypoxia on Differentiation of Metanephric Mesenchymal Stem Cells

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Objective: Effect of hypoxia on development is debatable. For embryonic kidney, it exposes in hypoxia environment at physiological condition. However, effect of hypoxia on development of kidney is unclear. The kidney (except the collecting duct) is derived from the metanephrogenic mesenchyme. Thus, we hypothesize that hypoxia in embryonic kidney is helpful to kept features of "stem cells" by inhibiting differentiation of metanephric mesenchyme stem cells (MMSCs). The aims of present work are to investigate the role of hypoxia in development of kidney by hypoxic organ culture; to determine the effect of hypoxia on differentiation of MMSCs and to explore roles of Wnt4/β-catenin pathway in hypoxia induced effect on MMSCs by hypoxic cell culture.
Methods: Embryonic kidneys or MMSCs were harvested from timed-pregnant female rats at embryonic days 13.5 or days 18 to 19 and cultured in normoxia (21% O_2) and hypoxia (3% O_2 for organ culture; 1% O_2 for cell culture). The number of ureteric buds and nephrons in embryonic kidneys were counted at 3 days after culture. As exposed to hypoxia, Wnt receptor of MMSCs was activated by LiCl (20 mM), then expression of member of Wnt4/β-catenin pathway including transcription factor LEF-1, downstream genes axin2 were measured.
Results: Number of ureteric buds and nephrons were decreased under hypoxia condition (Figure 1). Expression of Wnt4, β-catenin, LEF-1, axin2 were also decreased by hypoxia culture. LiCl treatment moderated the effect induced by hypoxia (Figure 2).

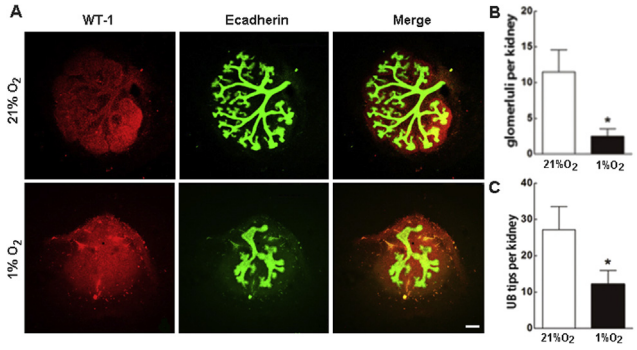

Fig. 1 Hypoxia inhibited development of embryonic kidney. A: Representative visual fields show glomerluli (red, WT-1 positive) and ureteric bud tips (green, E-cadherin positive) in normoxia and hypoxia cultured embryonic kidney. B and C: group data shows that number of glomerluli and ureteric bud tips were smaller in hypoxia cultured embryonic kidney (n = 8, *P < 0.05). Bar in A is 100μm.

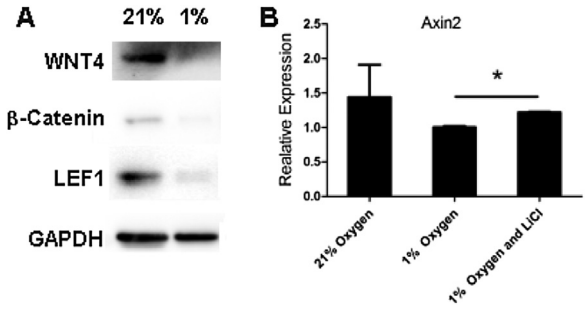

Fig. 2 Hypoxia inhibited WNT4/β-Catenin pathway in MMSCs. A: representative Western blots for WNT4, β-Catenin and LEF1, expression of them decreased in hypoxia cultured MMSCs. B: group data show that RNA level of Axin2 were decreased in hypoxia cultured MMSCs, LiCl moderated the effect induced by hypoxia (n = 3, *P < 0.05).

Conclusion: These data indicate that hypoxia suppressed differentiation of MMSCs by inhibiting Wnt signaling pathway and may affect the development of embryo kidney.

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Macrophage Myofibroblast Transition Contributes to Renal Fibrosis in Allograft Rejection

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Background: Chronic allograft rejection is a major complication following kidney transplantation. Inflammatory macrophage infiltration and progressive renal fibrosis are two major feature of chronic allograft rejection. Here we report that macrophage myofibroblast transition (MMT) plays critical role in the process of chronic renal allograft rejection in both experimental and human kidney transplantation.
Methods: MMT cells were identified by co-expressing macrophage ($CD68^+$ or $F4/80^+$ in mouse) and myofibroblast (α-SMA). To demonstrate MMT in patients with chronic allograft rejection, renal allografts from kidney transplant recipients (n = 32) were collected and analyzed by two-color confocal microscopy. To identify the MMT process in experimental chronic renal allograft rejection, a macrophage fate-mapping study in a mouse model of chronic kidney allograft rejection was induced by transplanting the donor kidneys from the BALB/c mouse into the LyZ^2-Cre/ Rosa-Tomato mouse (C57BL6). In addition, to study the signaling mechanism governing the MMT process, the donor kidneys from the BALB/c mouse was transplanted into the Smad3 knockout (KO) and wild type (WT) mice (C57BL6).
Results: We found that MMT cells as identified by $CD68^+\alpha\text{-SMA}^+$ cells were evidenced in patients with chronic renal allograft rejection, accounting for 30–40% of total $\alpha\text{-SMA}^+$ myofibroblasts. The number of MMT cells significantly correlated with total $\alpha\text{-SMA}^+$ myofibroblasts and progressive renal fibrosis. Similarly, $F4/80^+\alpha\text{-SMA}^+$ cells were also found the transplanted kidney with chronic allograft rejection in mice. The lineage tracing study showed that MMT cells (>90%) were presumably from bone marrow macrophages identified by expressing Tomato and are primarily derived from M2 type macrophages. More importantly, grafted kidneys from Smad3 KO recipients were protected from MMT, revealing a regulatory role for Smad3 in the process of MMT during chronic allograft rejection.